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Mitigating agricultural nitrogen load with constructed ponds in northern latitudes : A field study on sedimental denitrification rates

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1 **Mitigating agricultural nitrogen load with constructed ponds in northern latitudes: A field**
2 **study on sedimental denitrification rates**

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15
16 **Abstract**

17 Constructed agricultural ponds and wetlands can reduce nitrogen loading from agriculture especially
18 in areas where warm climate predominates. However, in cold climate temperature-dependency of
19 microbiological processes have raised the question about the applicability of constructed wetlands in
20 N removal. We measured *in situ* denitrification rates in a constructed agricultural pond using ¹⁵N-
21 isotope pairing technique at ambient light and temperature throughout a year as well as diurnally.
22 The field IPT measurements were combined with a wide set of potentially important explanatory data,
23 including air temperature, photosynthetically active radiation, precipitation, discharge, nitrate plus
24 other water quality variables, sediment temperature, oxygen concentration and penetration depth,
25 diffusive oxygen uptake and sediment organic carbon. Denitrification varied, on average, diurnally

between 12 and 314 $\mu\text{mol N m}^{-2} \text{ h}^{-1}$ and seasonally between 0 and 12409 $\mu\text{mol N m}^{-2} \text{ d}^{-1}$. Light and oxygen regulated the diel variation of denitrification, but seasonally denitrification was governed by a combination of temperature, oxygen and turbidity. The results indicated that the real N removal rate might be 30–35 % higher than the measured daytime rates, suggesting that neglecting the diel variation of denitrification we may underestimate N removal capacity of shallow sediments. We conclude, that by following recommended wetland:catchment -size ratios, boreal agricultural ponds can efficiently remove nitrogen by denitrification in summer and in autumn, while in winter and in spring the contribution of denitrification might be negligible relative to the loading, especially with short residence time.

Keywords

Denitrification, agriculture, *in situ*, sediment, nitrate, boreal

1. Introduction

Humans have transgressed the planetary boundaries in the fixation of N_2 (Rockström et al. 2009) by doubling the global amount of reactive nitrogen (N_r) (Fowler et al. 2013; Gruber and Galloway, 2008; Sutton et al. 2011). In Europe, the amount of N_r has been tripled and it is estimated that 40–70% of the fertilizer N_r applied for cereal production is lost to the environment (Sutton et al. 2011). Excess N leaching in receiving waterways has resulted in eutrophication and reduced water quality for drinking, agricultural, recreational, and other purposes (Galloway et al. 2013; Robertson and Vitousek, 2009). Only about half of the European surface waters met the Water Framework Directive (WFD) objective of good ecological status in 2015 and 25% of the ground waters investigated suffered from excess nitrate-N (NO_3^-) mainly caused by agriculture (EEA, 2015). Moreover, at the same time with the increased amount of N_r , the globe has lost more than half of its natural wetlands (Davidson 2014).

51

52 Constructed wetlands (CWs) have been successfully applied to remove excess nitrogen (N) from
53 agricultural runoffs (e.g. O'Green et al. 2010; Strand and Weissner, 2013; Vymazal 2017). CWs have
54 been shown to remove N in warm climates, but whether they work in cold conditions, has still been
55 called into question (Arheimer and Pers, 2017; Wang et al. 2018). In Finland, some non-remunerative
56 investments, e.g. agricultural CWs, are economically supported by the EU and national legislation,
57 and over a thousand wetlands have been built since 1995. Furthermore, the number of smaller pond
58 systems, e.g. individual or chains of sedimentation ponds is likely to be even greater. Agricultural
59 wetlands and ponds are often reasonably non-vegetated, due to highly turbid agricultural waters (e.g.
60 Tikkanen et al. 1985) limiting light penetration, and routine management practices like mechanical
61 excavation. Boreal sedimentation ponds are considered to exhibit insignificant and/or highly variable
62 N-retention, being more efficient in removing solids and phosphorus (P) (e.g. Häikiö 1998;
63 Vuollekoski et al. 2015). However, study reports from Sweden conclude that CWs can have a high N
64 removal, although it varies considerably (Strand and Weissner, 2013). Whether the reports of low N-
65 retention results from insufficient retention time, or negligible N removal processes in cold climate,
66 is poorly understood.

67

68 Three natural processes contribute to overall N retention in freshwater wetlands: denitrification,
69 sedimentation, and assimilation by aquatic biota. However, denitrification is the only pathway that
70 removes N entirely from the aquatic ecosystems. Denitrification is an anaerobic microbial process,
71 where nitrate nitrogen (NO_3^-) is reduced into gaseous form, either into nitrous oxide (N_2O) or nitrogen
72 gas (N_2) (Mitsch and Gosselink 2015). These gases are transferred into the atmosphere, balancing the
73 natural and anthropogenic N input. Denitrifiers utilize organic carbon (C) (heterotrophic
74 denitrification) or reduced inorganic compounds (e.g. sulfides; autotrophic denitrification) as energy
75 sources. Autotrophic denitrification is typical to marine environments (Shao et al. 2010), while

76 heterotrophic denitrification is considered to be the dominating process in freshwater ecosystems
77 (Mullholland et al. 2008).

78

79 Denitrification can be based on the NO_3^- from the water above the sediment (D_w), and/or from the
80 coupled nitrification-denitrification process (D_n), occurring in the oxic layers of the upper sediment.
81 In shallow sites with benthic primary production, higher sediment oxygen concentration followed by
82 increased photosynthetically active radiation (PAR) can promote D_n (An and Joye, 2001; Lorenzen
83 et al. 1998; Risgaard-Petersen et al. 1994), while D_w can be lower during light hours (Christensen et
84 al. 1990; Risgaard-Petersen et al. 1994). In boreal environments, where PAR amount changes
85 significantly between seasons (Lakkala et al. 2016), light-induced changes in the N removal of
86 shallow wetlands may be highly important. Besides changes in PAR and accompanying oxygen
87 conditions, temperature has been found to govern denitrification activity, explaining variable
88 denitrification rates in boreal lakes (Holmroos et al. 2012; Rissanen et al. 2011), temperate wetlands
89 (Bastviken et al. 2007; Hernandez and Mitch, 2007) and temperate stream sediments (de Klein 2008;
90 Veraart et al. 2014).

91

92 Current estimates of denitrification rates in agricultural wetland and stream sediments (e.g. Castaldelli
93 et al. 2015; Pinardi et al. 2009; Roach and Grimm 2011) are based on laboratory incubations
94 conducted in dark at constant temperature. Because of this the results do not necessarily reflect the
95 real, *in situ* denitrification rates. Moreover, sampling has been targeted only on certain seasons like
96 summer and on daytime. This paper reports *in situ* denitrification rates at ambient light and
97 temperature conditions in a constructed agricultural pond of northern Europe, Finland. The field
98 measurements were performed throughout the year, as well as diurnally. Using the direct ^{15}N -isotope
99 pairing technique (IPT, Nielsen 1992) simultaneously with sediment oxygen profiling, allowed us to
100 study the role of different environmental factors controlling denitrification rates at different temporal

101 and spatial scales. We expected ambient light and temperature regime being important drivers of
102 denitrification on annual basis in boreal agricultural ponds, which typically have high nutrient
103 concentrations throughout the year. Furthermore, we hypothesized that denitrification rates may also
104 show similar variation diurnally. On the basis of diel results, we recalculated the measured seasonal
105 N removal in the sediment of a boreal agricultural pond.

106

107 2. Materials and Methods

108 2.1 The catchment and the study site

109 The study was conducted in an agricultural watershed in southern Finland (61°04'97''N,
110 25°02'89'E) (Fig. 1). Koiransuolenoja is an approximately 4 km long brook flowing through typical
111 agricultural catchment (6.8 km²) into Lake Pääjärvi. The brook is approximately 1–2 m wide with an
112 average depth less than 0.5 m. The stream is heavily impacted by farming, as agricultural land covers
113 up to 24% of the drainage area (Arvola et al. 2015). Nearly half of the catchment surface area soil is
114 easily erodible material e.g. sand and silt (Tikkanen et al. 1985). During the study period (July 10th
115 2014–June 25th 2015), the average NO_x-N (indicating the NO₂⁻ plus NO₃⁻) concentration in the brook
116 was 194 μmol l⁻¹ (SD ±58 μmol l⁻¹, n=48).

117

118 An agricultural sedimentation pond had been built one year earlier in March 2013. Aquatic vegetation
119 had not yet developed into the littoral, and no shading was provided by trees or shrubs. Surface area
120 of the pond was 320 m² and volume 226 m³ (mean depth 0.7 m., max. depth 1.6 m). Average discharge
121 in Koiransuolenoja was 0.058 m³ s⁻¹ resulting in theoretical residence time approximately one hour
122 in the pond. The real residence time varied from 15 minutes to 4.5 hours depending on the discharge.
123 Water flow in different parts of the pond was measured with a flow meter (MiniAir2, Schiltknecht)
124 and the flow rate in the pond littoral was 0 m s⁻¹ on each experiment date throughout the study. We
125 investigated the grain size of the homogenized and dried (48h, 60 °C) top sediment (0–3 cm) with a

126 vibratory sieve shaker (Analysette 3, Fritsch, Germany). The shallow littoral where denitrification
127 was measured (Fig. 1) consisted of accumulation sediment having the highest amounts (91%) of fine
128 materials, fine sand (grain size 0.063–0.125 mm) and silt (0.002–0.062 mm). In the deepest part of
129 the pond, 64% of the top sediment was fine materials. Two species of non-nitrogen-fixing benthic
130 algae *Spirogyra* and *Planktothrix* sp. was almost every time observed on the sediment surface.

131

132 2.2 Sampling, field and laboratory analyses

133 Sediment samples and water NO_x-N samples for denitrification measurements were taken manually
134 from the littoral zone of the constructed pond (Fig. 1). Quality of the stream water (turbidity, dissolved
135 organic carbon (DOC), NO_x-N, ammonium (NH₄⁺-N) and total N (TN)) was investigated weekly
136 from water samples taken at site K1 (Fig. 1), representing the water quality at catchment scale.
137 Discharge was calculated from the discharge curve based on the measured water level and flow rate.
138 Data for air temperature and precipitation measured at Lammi biological Station (Fig. 1), 800 m NE
139 from the study site, were obtained from The Finnish Meteorological Institute. Photosynthetically
140 active radiation (PAR) was measured with a quantum sensor (PQS1, Kipp & Zonen) at 10-min
141 intervals, located 600 m NE from the study site (Fig. 1). In laboratory, NO_x-N, NH₄⁺-N, and TN
142 concentrations were analyzed using standard laboratory methods (see Arvola et al. 2015). Samples
143 for DOC were filtered through pre-rinsed cellulose ester filters (pore size 0.45 µm, Millex-HA, Merck
144 Millipore) and analyzed using a carbon analyzer (Ordior TOC-V, Shimadzu). Organic C content of
145 the study littoral sediment (0–3 cm) was calculated from the loss on ignition (LOI%) of oven dried
146 material (550 °C, 2 h). Temperature was measured from the top of the sediment using the flow meter.
147 Oxygen (O₂) penetration depth in the sediment (OPD) and O₂ concentration on top of the sediment
148 were measured with clark-type microelectrode (tip Ø 100 µm) in the laboratory from three replicate
149 intact sediment-water cores within one hour of sampling (OX100-sensor, PA-2000, Unisense).
150 Sediment diffusive O₂ uptake (DOU) was calculated from the flux through the diffusive boundary

151 layer above the sediment from the oxygen profiles (Jørgensen and Revsbech, 1985; Revsbech et al.
152 1980).

153 154 2.3 Sediment core incubations and isotope analysis for denitrification

155 Before the field incubations, the validity of the method at the study site (independency of
156 denitrification of ambient NO_x-N and positive dependency of denitrification of ¹⁵N-labeled nitrate on
157 the added tracer amount) and the possible presence of anammox (Nielsen 1992, Risgaard-Petersen et
158 al. 2003), was investigated in a pre-experiment with a concentration series in the laboratory using
159 ¹⁵N-labeled potassium nitrate (50, 100, 200, 400, and 600 μmol of K¹⁵NO₃, 98 atom %, Sigma-
160 Aldrich).

161
162 Denitrification experiments were performed at the pond littoral at water depth of 30–40 cm. Sediment
163 cores were incubated *in situ* under ambient light and temperature conditions, using ¹⁵N-isotope
164 pairing technique (IPT) by Nielsen (1992). Diel denitrification rates were measured twice, on the 28th
165 and 25th of August in 2014 and 2015, respectively. Three undisturbed replicate sediment samples
166 were collected in transparent plastic cores (height 16 cm, diam. 2.6 cm) so that each core contained
167 1/3 of sediment and 2/3 water. Both 24 h denitrification experiments were divided into eight 3-hour
168 incubation periods. Seasonal denitrification was measured during daytime, between 10:00 and 14:00,
169 24 times between July 7th 2014 and June 25th 2015 (except not in January and February 2015) using
170 three replicate sediment cores (height 34.2 cm, diam. 4.1 cm). For each 3 h period in the 24 h
171 experiments and in the seasonal experiment, OPD and O₂ concentration on the top of the sediment
172 was measured with the microprofiler, and DOU was calculated. Since we were not able to use stirring
173 during the field incubations, there might have been some oxygen stratification, decreasing OPD and
174 affecting the ratio between D_w and D_n. The average NO_x-N in the pond littoral water was based on
175 two samples during each 24 h experiment, due to the limited accuracy of the NO_x-N analysis (±10 %

176 in concentrations $>21 \mu\text{mol l}^{-1}$). To test the immediate effect of PAR on daytime denitrification, three
177 replicate all-covered, dark cores were incubated simultaneously on two occasions in the seasonal
178 study: May 13th under high turbidity (36.3 ftu) and May 26th under low turbidity (7.0 ftu).

179

180 Based on the pre-experiment in the laboratory, concentration of $200 \mu\text{mol K}^{15}\text{NO}_3$ was used in
181 labeling in the field experiments. After labeling, the water column was gently mixed with a glass rod
182 to ensure complete mixing of the label to the water phase, not disturbing the sediment. The three
183 labeled cores were sealed tightly, pushed back into the original sediment depth, and incubated for 3
184 h. To avoid changes in sediment oxygen conditions, metal foil cover was adjusted to the height of the
185 sediment surface to prevent the deeper layers of the sediment being exposed to sunlight during
186 labeling in the seasonal study. One unlabeled core was always used as a control for ambient N_2
187 concentration. After the incubation, the sediment and water were mixed into a slurry in the core and
188 let settle for five minutes, before transferring a 12-ml subsample with a syringe and a gas-tight Tygon-
189 tube into a glass vial (Exetainer 12 ml 738W, Labco Limited). One hundred μl of formaldehyde
190 solution (37 wt%, Sigma-Aldrich) was added to terminate all microbial activity. Subsamples were
191 stored upside down ($+4^\circ\text{C}$, in dark) until N isotope analysis. A helium headspace was added to each
192 sample before the isotope analysis, following Tirola et al. (2011). The isotope mass-area -ratios (m/z
193 28, 29, and 30) and N_2 concentration of the samples were analyzed with Isoprime IRMS connected
194 to a Tracegas preconcentrator unit, using a modified N_2O project with no cryotrapping, and valves in
195 CO_2 mode. Total denitrification, D_w and D_n were calculated as in Rissanen et al. (2013).

196

197 2.4 Data analysis

198 For diel data, we used paired t-test and Wilcoxon rank test to identify potential differences in
199 denitrification and environmental factors during light and dark. General linear model (GLM) was
200 used to explore the relationship of the denitrification rates to environmental variables, including

201 temperature, O₂ concentration, DOU, OPD, and PAR. Due to data skewness, denitrification rates
202 were LN-transformed in GLM. To control the non-independency of observations arising from the
203 similarity of the sampling site, year was added to the models as a categorical variable. Akaike
204 information criterion (AIC) was used for selecting the minimum adequate model. Model selection
205 was carried out using the step AIC function from R package “MASS” (Venables and Ripley, 2002).
206 To test whether the main effect of the categorical variable ‘year’ was significant, GLM models with
207 and without categorical variables were compared with log-likelihood tests.

208

209 For seasonal data, we used a model describing the interactions between environment and
210 denitrification. First, we performed correlation and head component analysis between the catchment
211 scale factors (average air temperature, 2-day temperature sum, 2-day rain sum and daily PAR sum as
212 well as discharge, turbidity, pH, DOC, TN, NO_x-N, NH₄⁺-N from K1), process scale factors (sediment
213 temperature, DOU, O₂ concentration on the sediment surface, O₂ penetration depth, PAR sum during
214 incubation, NO_x-N in the water above the sediment, top sediment LOI%) and denitrification.
215 Secondly, we performed structural equation modeling (SEM, e.g. Sutton-Grier et al. 2010) using the
216 R package ‘lavaan’. A chi-square test of model fit (P-value >0.05) was used for indicating that the
217 model and data structure do not differ significantly. In addition, comparative fit index (CFI) >0.95
218 and root mean square error of approximation (RMSEA) <0.05 were used in model estimation.
219 Significance for individual path coefficients was accepted below 0.05. Path coefficients in the SEM
220 models were standardized against their standard deviation and express the approximate change of
221 observed range. Thus, the approximate strength of various paths should be cautiously compared. It
222 should be noted, that the three replicate denitrification samples might not be independent from each
223 other, due to the same sampling site, thus violating the statistical requirements of true replicates.
224 Consequently, the P-values should be considered with caution. Statistical analyses were performed
225 with SPSS 24 (IBM) and R (R Development Core Team 2016).

226

227 Finally, we estimated the influence of the length of daylight through oxygenation on denitrification.

228 We calculated the weighted average on the basis of measured denitrification rates in dark and in light

229 combining them to the light/dark hours from the study days based on PAR data.

230

231 3. Results

232 3.1 Environmental conditions during seasonal sampling

233 Pre-experiment with a concentration series of $^{15}\text{NO}_3\text{-N}$ showed that denitrification was limited by

234 $\text{NO}_x\text{-N}$ (ambient $\text{NO}_x\text{-N}$ $163\ \mu\text{mol l}^{-1}$), as the denitrification of labeled $\text{NO}_x\text{-N}$ (D15) increased

235 relative to the concentration of labeled $\text{NO}_x\text{-N}$ (Fig. 3). Also, denitrification was the only process

236 producing N_2 , because denitrification based on the natural $\text{NO}_x\text{-N}$ (D14) was stable in spite of the

237 increased labeled $\text{NO}_x\text{-N}$ (Fig. 3), and the conclusion was that anammox was not present. The

238 presence of anammox would have resulted in increasing D14 relative $^{15}\text{NO}_3\text{-N}$ amendments due to

239 the production of $^{28}\text{N}_2$ ja $^{29}\text{N}_2$ gases.

240

241 During the study days, the temperature on the sediment surface was above the freezing point. The

242 minimum was measured in late October ($+0.1\ ^\circ\text{C}$) and the maximum ($+17.2\ ^\circ\text{C}$) in July (Fig. 2A).

243 During winter the sediment temperature was higher than the air temperature (Fig. 2A). The lowest

244 OPD and O_2 concentration were measured in summer on the sediment surface in the first year (Fig.

245 2B). In this agricultural watershed, the N consisted mostly of $\text{NO}_x\text{-N}$ (Fig. 2D). The average $\text{NO}_x\text{-N}$

246 concentration in the pond littoral water was $167\ \mu\text{mol l}^{-1}$ following the concentrations in the incoming

247 water at K1 ($87\text{--}388\ \mu\text{mol l}^{-1}$). The maximum $\text{NH}_4^+\text{-N}$ concentration ($36\ \mu\text{mol l}^{-1}$; data not shown)

248 was measured on July 30th 2014 and was most likely connected to the spread manure in the fields and

249 the heavy rain that occurred the next day. The highest stream discharge was measured after spring

250 melt 2015 simultaneously with heavy rain (Fig. 2C). In the spring, average pH was 6.9 and during

251 other seasons 7.3. Average discharge of Koiransuolenoja was 58 l s^{-1} , varying between 5–200 l s^{-1} .
252 Highest turbidity and DOC concentrations occurred after the rain. Average LOI of the upper sediment
253 in the pond varied between 7.3–9.8%.

254

255 3.2 Diel denitrification

256 In field incubations, O_2 concentration on the sediment surface was lower during dark ($\text{PAR} < 1 \text{ } \mu\text{mol}$
257 $\text{s}^{-1} \text{ m}^{-2}$) than during light (Table 1, Fig. 4). The maximum temperature on the sediment surface was
258 measured at 18:00 and the minimum between 03:00–06:00 at night. Denitrification rates were driven
259 by light conditions and varied between the two study occasions (Table 2). Average denitrification
260 rates during dark were significantly higher ($180 \text{ } \mu\text{mol N m}^{-2} \text{ h}^{-1}$) than during light ($67 \text{ } \mu\text{mol N m}^{-2} \text{ h}^{-1}$)
261 Table 1, Fig. 4). In 2015, average denitrification was nearly two-fold ($142 \text{ } \mu\text{mol N m}^{-2} \text{ h}^{-1}$) as
262 compared to the previous year ($77 \text{ } \mu\text{mol N m}^{-2} \text{ h}^{-1}$). Also, in 2015 the water above the sediment had
263 higher $\text{NO}_x\text{-N}$ concentration and sediment temperature ($138 \text{ } \mu\text{mol l}^{-1}$, $14.3 \text{ } ^\circ\text{C}$) than the previous year
264 ($112 \text{ } \mu\text{mol l}^{-1}$, $12.6 \text{ } ^\circ\text{C}$).

265

266 3.3 Seasonal denitrification

267 Maximum denitrification rate ($12409 \text{ } \mu\text{mol N m}^{-2} \text{ d}^{-1}$) took place during the summer (July 30th; Fig.
268 5). At the time, sediment surface O_2 and littoral water $\text{NO}_x\text{-N}$ concentration were the lowest (Fig. 2B
269 and D) after a heavy rain and sediment temperature was the second highest measured ($+15.8 \text{ } ^\circ\text{C}$).
270 Daytime *in situ* denitrification rates clearly decreased in sediment temperatures below $+10 \text{ } ^\circ\text{C}$, and
271 no activity was detected during the minimum sediment temperature $+0.1 \text{ } ^\circ\text{C}$ in October (Fig. 6).
272 Annual average denitrification was $2441 \text{ } \mu\text{mol N m}^{-2} \text{ d}^{-1}$, consisting mainly of the denitrification of
273 the water column $\text{NO}_x\text{-N}$ (66%). Fall was the only season when D_n (nitrification-denitrification)
274 slightly dominated over D_w by 53% (Table 3).

275

276 Turbidity level affected the daytime denitrification rates, because under high turbidity (36.3 ftu),
277 denitrification was 19% higher in the covered cores than in the transparent ones (Fig. 7), but during
278 clear water (7.0 ftu), denitrification was 68% higher in the covered cores than in the transparent ones.
279 The seasonal model explained 40% of the denitrification variability, 4% of the sediment surface
280 temperature variability and 60% of the sediment surface O₂ variability (Fig. 8). Season (represented
281 by the 2-day temperature sum) regulated sediment surface temperature and higher turbidity and
282 temperature led to lower O₂ concentration on the sediment surface.

283

284 3.4 Recalculated denitrification rates

285 On the basis of light/dark correction of denitrification rate, average annual estimate of denitrification
286 was 3415 $\mu\text{mol N m}^{-2} \text{ d}^{-1}$, being 40% higher than the measured daytime denitrification (Fig. 5).
287 Compared with measured seasonal averages in Table 3, recalculated denitrification rates were 35%
288 higher in summer, 71% higher in fall, 137% higher in winter and 31% higher in spring.

289

290 4. Discussion

291 Following our hypothesis, the diel measurements in August 2014 and 2015 showed that
292 denitrification rates varied significantly between night and day. Our result corroborates with previous
293 findings from freshwater systems, where highest denitrification rates were measured in dark
294 conditions (Christensen et al. 1990; de Klein 2008; Soana et al. 2017). Higher PAR during the day
295 promotes O₂ production on the sediment surface by the benthic algae, pushing the denitrification zone
296 deeper (e.g. Christensen et al. 1990; Laursen and Carlton, 1999; Nielsen et al. 1990). Consequently,
297 nitrate in the above water needs to diffuse longer path to reach the denitrifying bacteria in the anoxic
298 sediment layers. Indeed, we found D_w being lower when OPD was higher, which is in line with earlier
299 results (Andersen et al. 1984; Christensen et al. 1990; Risgaard-Petersen et al. 1994). Compared to

300 sediment surface O₂ concentration, sediment temperature remained more stable throughout the 24h
301 experiments. Higher denitrification rate observed in 2015 than 2014 in the diel studies is probably
302 linked to higher NO_x-N availability, but also to other factors combined such as higher sediment
303 temperature, thinner oxygenated sediment layer, and thus, higher D_w.

304

305 Denitrification rates showed clear seasonal variation, which was driven by temperature, oxygen and
306 turbidity. The denitrification rates were clearly accelerated in sediment temperatures above +10 °C,
307 but the summer was the only season when the average sediment temperature reached this high.
308 Seasonal denitrification was more related to air temperature (2-day air temp. sum) than to PAR. The
309 seasonal study measurements were conducted on daytime and we used PAR data merely from the
310 incubation time (3h), which may be the reason why air temperature explained more about the variation
311 in denitrification than PAR. The 2-day air temperature reflects longer term thermal conditions in the
312 watershed, and apparently also reflects sediment temperature slightly better than PAR. However, air
313 temperature explained only 4% of the sediment temperature variability of the study site. This can be
314 explained by the on-stream nature of the pond, where different discharge situations are reflected into
315 temperature of the sediment.

316

317 Temperature and turbidity mediated denitrification indirectly by modifying sediment O₂
318 concentrations. However, turbidity also directly controlled denitrification in seasonal results, which
319 can refer to higher C availability or lower O₂ concentration (Liu et al. 2013) of turbid waters.
320 Turbidity is typical in agricultural streams and reduces light reaching the stream bottom. The highest
321 denitrification rates coincided with heavy rains washing out the liquid manure spread on the fields to
322 the stream increasing turbidity and C:N-ratio in the stream water. Carbon in the organic manure was
323 likely more labile to the denitrifiers as compared to the terrestrial C typically leaching from the
324 catchment area. The importance of C quantity and lability for denitrifiers has been addressed in

325 several studies (e.g. Asmala et al. 2013; Grebliunas and Perry, 2016; Stelzer et al. 2014).
326 Denitrification based on water $\text{NO}_x\text{-N}$ was high (81%) at the time, indicating the denitrification was
327 not based on the $\text{NO}_x\text{-N}$ produced in the sediment. In this study, $\text{NO}_x\text{-N}$ concentrations did not
328 regulate the denitrification rates, which was presumably a result of the high $\text{NO}_x\text{-N}$ level in the stream.
329 Considering spatial variability in the pond sediment denitrification (Uusheimo, unpublished), we
330 observed 1.7 times higher rates in the deepest part of the pond compared to the littoral. These
331 laboratory experiments were conducted six times during the year in dark at constant temperature with
332 the same method resulting mean denitrification rates of ca. $5400 \mu\text{mol N m}^{-2} \text{ d}^{-1}$, while in the littoral
333 sediment the rate was ca. $3100 \mu\text{mol N m}^{-2} \text{ d}^{-1}$. This difference may be explained by the higher C
334 content and lower oxygen conditions of the deep sediment. The observed patchiness also indicates
335 that even our recalculated rates from the littoral sediment underestimate N removal and should be
336 regarded as the minimum in these conditions.

337

338 It is challenging to compare our measured seasonal daytime denitrification results ($0\text{--}12409 \mu\text{mol N}$
339 $\text{m}^{-2} \text{ d}^{-1}$) to other freshwater studies in the boreal region due to the scarcity of actual denitrification
340 rate measurements and especially because of the differences in the methods applied. Isotope pairing
341 technique has been applied for measuring denitrification rates only in a few boreal lakes in Finland
342 (Holmroos et al. 2012; Rissanen et al. 2013) and in Sweden (Ahlgren et al. 1994), but not in boreal
343 streams or constructed agricultural ponds. The rates measured in our study were significantly higher
344 as compared to the results from boreal lakes ($0\text{--}2070 \mu\text{mol N m}^{-2} \text{ d}^{-1}$). One reason may be that even
345 the most eutrophic lakes have lower $\text{NO}_x\text{-N}$ concentration and temperature than agricultural wetlands.
346 Similar or higher denitrification rates using IPT have been reported in studies conducted in temperate
347 nitrate-rich aquatic systems (e.g. de Klein 2008; Racchetti et al. 2011; Soana et al. 2017).

348

349 Recalculated denitrification rates, based on the diel study, indicated that daytime denitrification
350 measurements can lead to substantial underestimations of denitrification rates. For example, the
351 measured denitrification was 35 % smaller than the light-corrected rate in summer. This can be
352 explained with day-time top sediment oxygenation decreasing the denitrification based on D_w , which
353 is usually the dominant denitrification pathway in systems with high nitrate level. In fall, our corrected
354 estimate was 71% higher than the measured one. We acknowledge that this estimate may be too high,
355 since denitrification was mostly based on coupled nitrification-denitrification fed by active
356 mineralization during fall. Furthermore, during the one study date at air temperature below zero in
357 winter, the darkness lasted for 17 hours which explains the large proportional difference between the
358 measured and the recalculated denitrification. We emphasize that this kind of correction can be
359 applied in sites where denitrification is based mostly on water nitrate, to form a proxy of the influence
360 of light reaching the sediment primary producers and thus, denitrification.

361

362 In the agricultural pond sediment, the highest denitrification rates were measured during the growing
363 season. During other seasons, denitrification rate was slower, but detectable still at sediment
364 temperature +0.4 °C, at the time the air temperature had fell to -6.2 °C. This indicates some microbial
365 activity in the sediment throughout the winter. Denitrification rates might have been underestimated
366 in winter, since higher oxygen penetration depth could have led to inhomogenous mixing of natural
367 and labeled nitrate or slow nitrate diffusion rate into the sediment denitrification zone (Nielsen 1992).
368 Low denitrification rates can be compensated to some extent by longer retention time (e.g. Addy et
369 al. 2016). In a literature review by Leonardson (1994), retention time of 3–5 days was recommended
370 for N removal at times of high discharge. One of the main reasons sedimentation ponds are not
371 considered to be applicable to N removal is typically their small surface area relative to the catchment
372 size, and small volume relative to discharge, and thus, a short retention time counted in hours (e.g.
373 Puustinen et al. 2007).

374

375 The seasonal nitrogen removal of small Koiransuolenoja pond was not capable of corresponding to
376 the high N loading from the catchment, although the actual denitrification rates in the sediment were
377 high, because the average seasonal residence time was relatively low varying from 0.5 hours to 2.3
378 hours. The mean nitrate load in the stream was 4.4 kg d⁻¹ in summer, 5.6 kg d⁻¹ in fall, 11 kg d⁻¹ in
379 winter, and 40 kg d⁻¹ in spring, meaning denitrification could remove only 0.8% of incoming nitrate
380 in summer and 0.08% in fall. Recommendations stating a sufficient wetland:catchment -size ratios
381 for efficient nutrient removal vary from 0.5% (Puustinen et al. 2007) to 2% (Hammer 1992). In our
382 study pond, this ratio was only 0.005%, meaning that a notable increase in the wetland area is needed,
383 if we want to remove a major proportion of the annual nitrate loading by microbial processes. With
384 the seasonal recalculated denitrification rates in the shallow littoral, representing the lower end in the
385 spatial variation in denitrification rates, and wetland:catchment -size ratio of 2% suggested by
386 Hammer (1992), nitrate removal by denitrification in Koiransuolenoja pond would be 100% in
387 summer, 32% in fall, 4.7% in winter and 5.2% in spring, respectively. Furthermore, with the
388 wetland:catchment -size ratio of 6%, nearly all (95%) of the nitrate load could be removed also during
389 the fall, but the efficiency during winter and spring would be only 15%. Thus, not only the
390 denitrification rates, but also residence time should be taken into account when defining the sufficient
391 surface area and volume for the wetland to remove nitrate efficiently also in winter and spring.

392

393 5. Conclusions

394 Nitrogen removal capacity of shallow wetlands can be underestimated due to day-time top sediment
395 oxygenation, if the potential is solely based on day-time denitrification measurements. Seasonally,
396 denitrification was controlled by temperature, which makes N removal challenging in boreal latitudes
397 during cold seasons. In the warming climate, efficiency and importance of constructed wetlands will
398 increase, as precipitation and nitrogen load have been estimated to rise. Mitigating agricultural

399 nitrogen load in northern latitudes requires wetlands large enough, relative to their catchment size,
400 and elaborated plans for restoring watersheds to utilize and accelerate their ecosystem services,
401 including denitrification.

402

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407

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557 **Table 1** Pairwise comparisons of denitrification rate, oxygen (O₂) concentration on sediment surface,
 558 sediment diffusive O₂ uptake (DOU) and temperature, photosynthetically active radiation (PAR), O₂
 559 sediment penetration depth and denitrification based on the water column nitrate (D_w%) between light
 560 (09:00–18:00) and dark conditions (21:00–06:00) in the diel studies. Degrees of freedom (df), test
 561 values (t) and significance (P-value) of the results.

	df	t	P-value
Denitrification rate	16	-7.639	<i><0.001</i>
O ₂ concentration	14	6969	<i><0.001</i>
DOU	14	0.289	0.78
Temperature	17	0.14	0.99
PAR	18	0*	<i><0.05*</i>
O ₂ penetration depth	15	35.5	0.165*
D _w %	11	32	0.928*

Statistically significant results (P<0.05) are shown in italics.
 *) Wilcoxon signed rank test for data with non-normally distributed variance.

562

563 **Table 2** General linear model (GLM) results for denitrification in relation to the environmental
 564 factors: temperature and oxygen (O₂) concentration on sediment surface, sediment diffusive O₂
 565 uptake and photosynthetically active radiation (PAR) in the diurnal experiments. Estimates, standard
 566 error, test values (t) and significance (P-value) of the results.
 567

		estimate	std. error	t	P-value
Denitrification AIC 21.25	intercept	7.05	1.56	4.53	0.001
	Year			0.59	<i>0.044</i>
	Temperature	0.21	0.13	1.66	0.127
	O ₂ concentration	-0.23	0.12	-1.91	0.086
	DOU	-0.32	0.19	-1.74	0.112
	PAR	-0.001	0.0004	-2.97	<i>0.014</i>

568 Statistically significant (P<0.05) results are shown in italics.
 569

570 **Table 3** Seasonal averages of the data July 10th 2014–June 25th 2015: Measured and estimated
571 denitrification, denitrification based on the water column NO_x-N (D_w%), sediment temperature, O₂
572 concentration, O₂ depth (OPD), diffusive O₂ uptake (DOU), loss on ignition (LOI%, 0-3 cm) and
573 NO_x-N above the pond littoral sediment. NO_x-N, turbidity, dissolved organic carbon (DOC) and
574 discharge at K1 as well as photosynthetically active radiation (PAR) and air temperature.

Seasons	Summer	Fall	Winter	Spring
Months	(VI–VIII)	(IX–XI)	(XII–II)	(III–V)
Study days included	9	8	1	6
Meas. denitrification (μmol N m ⁻² d ⁻¹)	5520	576	120	864
Est. denitrification (μmol N m ⁻² d ⁻¹)	7444	984	285	1135
D _w (%)	69	47	88	71
Sediment surface temp. (°C)	14	7	0	5
Sediment surface O ₂ (μmol l ⁻¹)	182	298	218	265
Sediment OPD (mm)	5	7	11	7
Sediment DOU (mmol O ₂ m ⁻² d ⁻¹)	5	4	2	5
LOI (%)	9	8	8	8
NO _x -N above sediment (μmol l ⁻¹)	131	135	190	256
NO _x -N at K1 (μmol l ⁻¹)	135	128	156	251
Turbidity (ftu)	16	10	15	16
Water DOC (mg C l ⁻¹)	10	10	8	11
Discharge (l s ⁻¹)	27	36	58	130
PAR (mmol m ⁻² s ⁻¹)	592	218	1	187
Air temp. (°C)	17	6	-6	5

575